

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

**(19) World Intellectual Property Organization  
International Bureau**



A standard linear barcode is located at the bottom of the page, spanning most of the width.

**(43) International Publication Date  
21 February 2002 (21.02.2002)**

PCT

**(10) International Publication Number  
WO 02/14535 A2**

(51) International Patent Classification<sup>7</sup>: C12Q 1/00

(21) International Application Number: PCT/GB01/03615

(22) International Filing Date: 13 August 2001 (13.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0019694.9            11 August 2000 (11.08.2000) GB

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

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WO 02/14535 A2

(54) Title: ELECTROCHEMICAL STRIP TEST FOR SMALL VOLUMES

**(57) Abstract:** A device for the electrochemical detection of an analyte in a sample, comprises electrodes and appropriate reagents on a substrate defining a volume within which the sample can be detected, wherein said volume is 500 nL or less.

## ELECTROCHEMICAL STRIP TEST FOR SMALL VOLUMES

### Field of the Invention

This invention relates to an electrochemical test cell for the measurement of reactive species. Such a sensor can be used for the measurement of enzymes, enzyme substrates, cofactors or mediators. One particular application is the measurement of glucose for use in diabetes.

### Background of the Invention

Diabetes mellitus is the most common endocrine disease and affects between 3-8% of the population. Various studies, such as the UK Prospective Diabetes Study (UKPDS, Lancet, 12th September 1998) have pointed out the importance of maintaining a normal glucose level in the blood or plasma, in order to delay the onset of diabetic complications.

Many diabetics monitor their disease by a regime of tests at home. A simple means of monitoring their disease is therefore desirable.

Electrochemical strip tests for glucose, electrolytes, lipids and enzymes have all been widely discussed in the patent and other literature. For example, US-A-4224125 discloses an enzyme electrode system in which an enzyme such as glucose oxidase is used to sense glucose. US-A-5820551 discloses such a strip electrode fabricated by screen printing.

### Summary of the Invention

The present invention is based on the realisation that, by using appropriate chemistry etc, an adequate signal can be achieved if a cavity is provided that is smaller than has previously been used, i.e. which holds 500 nL or less of blood. In particular, the invention allows the detection of glucose in whole blood, using a simple to use strip test, characterised in that the volume of sample required is very small, e.g. less than 200 nL and down to 50 nL.

In most respects, a device of the invention can be made from known materials. However, it has been found that its size can be reduced, because sufficient current density can be achieved that there is adequate sensitivity for the analyte, typically 90-100 nA/mM glucose.

The small volume of blood that can be utilised in the invention means that the sample can be taken from a part of the body having a low vasculature. Thus, for example, the sample can be taken from the arm.

Brief Description of the Drawings

5 Figures 1 to 3 of the drawings are each associated plan and side views of different illustrative embodiments of the invention. The drawings show a cell or sample area 1, a cover 2, a spacer layer 3, a reference/counter electrode 4, a working electrode 5, a cell-defining layer 6, a counter electrode 7, a reference electrode 8, and a strip/sample electrode 9.

10 Description of the Invention

Electrochemical strips for the detection of glucose in whole blood typically comprise an working, reference and counter electrode, or combination thereof, for the detection and quantitation of the activity of reagents or reaction products on the strip. Examples of these reagents may include enzymes, enzyme products or mediators accepting electrons from redox enzymes on the strip.

15 The electrodes are preferably printed or deposited onto an elongate substrate, configured for ease of use by the diabetic and for insertion into a reader. Such a substrate may also include other tests of use for the diabetic such as, but not limited to, glycosylated haemoglobin, glucose and potassium.

20 Some of the reagents may be deposited directly onto the electrodes themselves or onto other structures incorporated in the device, including wetting meshes such as is known in the art. The mesh is preferably as described in WO-A-00/28068. The use of a mesh in the cavity, e.g. having an open area of about 30%, can reduce the sample volume.

25 Appropriate reagents may be placed on the electrochemical strip test by methods including, but not limited to, screen printing and dosing the enzymes or enzyme-mediator couples. Especially for the detection of glucose, such reagents may be, for example:

glucose oxidase and ferrocene/ferricyanide

30 glucose oxidase and oxygen to yield hydrogen peroxide

glucose dehydrogenase and ferricyanide

glucose dehydrogenase and NAD and Meldola's Blue

The effect of interferents in the test may be reduced by including enzymes or chemicals to reduce ascorbate interference, e.g. ascorbate oxidase. Haematocrit interference may be reduced by including saponin, digitonin, or other lysing agents.

5 PVP, PVA and other polymers may be included, to increase linearity. Surfactants may be added to the device, to aid filling.

The assay may also incorporate features such as filling via the side of the elongate substrate, to assist the diabetic in using the device. This is described in WO-A-00/28086 and also WO-A-98/55856 (the contents of both of which are 10 incorporated herein by reference).

The output from the device may also be quantitated on small electronic instrument. Analysis of the output from the device may be *via* coulometry, amperometry, analysis or cyclic voltamogram or other electrochemical analytical methods known in the art.

15 The arrangement of the device may include provision for the sequential exposure of reagents to the sample, to allow the reactions to proceed in a sequential or time-delayed manner, to optimise the readout.

In a typical configuration that embodies the invention, the device comprises two electrodes placed approximately 100 µm or more apart: a 20 counter/reference electrode preferably made of Ag/AgCl and a second conductor of either Au, Pt, Pt/C or C. A well is constructed by laminating a plastics layer around the cell or printing a layer around the cell, as shown in Figure 1. A top cover may then be added to complete the cell. The electrodes are approximately 100 µm wide and 1000 µm long. Before laminating the top cover onto the 25 system, the reagents are dosed onto the device. Strips made with a Au, Pt or Pt/C conductor may contain glucose oxidase. Hydrogen peroxide is detected electrochemically on the strip. Strips made of a carbon conductor may contain a redox enzyme (glucose oxidase or glucose dehydrogenase) and a mediator for the enzyme (potassium ferricyanide or phenoxazines including Meldola's Blue).

30 In a second embodiment of the invention, a device comprises 3 conducting electrodes, namely a working electrode (carbon, Pt, Au or Pt/C), a reference electrode made of Ag/AgCl and a counter electrode (C, Pt, Au or Pt/C),

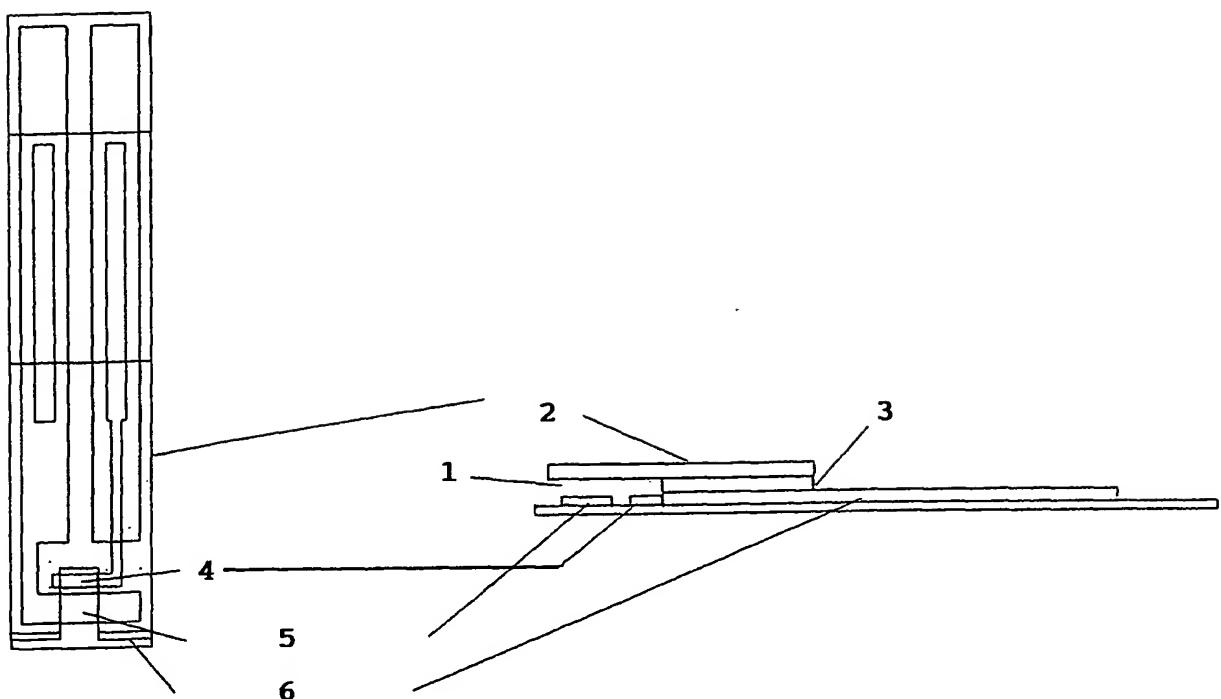
as shown in Figure 2. The reagents, i.e. enzyme, cofactor and mediator, are deposited on the working electrode as a single or multiple layers. Alternatively, the reagents are dried over the whole of the cell. A channel is created over the reagents by laminating a thin plastic layer or printing a layer of adhesive around 5 the cell. A clear cover with an air exit hole is placed over the device to complete the test strip. The cell volume can be further reduced by sandwiching a mesh layer between the substrate containing the conducting electrodes and reagents and the cover.

In a third embodiment of the invention, a device comprises 10 3 conducting electrodes, namely a working electrode (carbon, Pt, Au or Pt/C), a reference/counter electrode made of Ag/AgCl and a strip/sample detection electrode (C, Pt, Au or Pt/C), as shown in Figure 3. The reagents, i.e. enzyme, cofactor and mediator, are deposited on the working electrode as a single or multiple layers. Alternatively, the reagents are dried over the whole of the cell. 15 A channel is created over the reagents by laminating a thin plastic layer or printing a layer of adhesive around the cell. A clear cover with an air exit hole is placed over the device to complete the test strip. Again, the cell volume can be further reduced by sandwiching a mesh layer between the substrate containing the conducting electrodes and reagents and the cover.

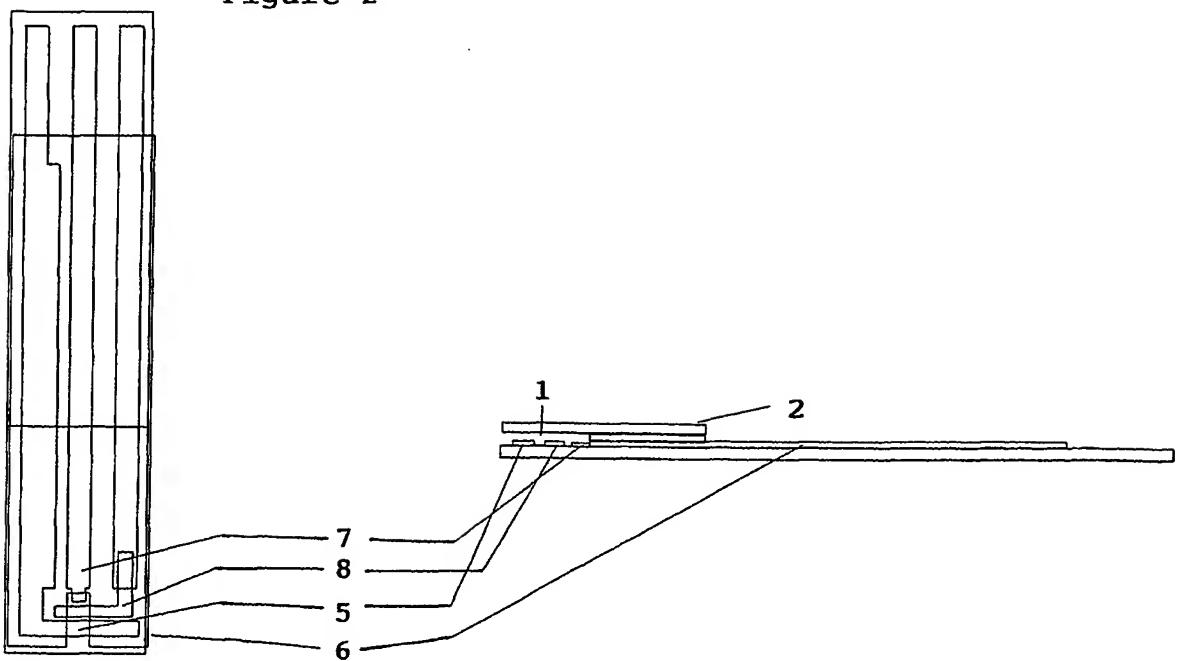
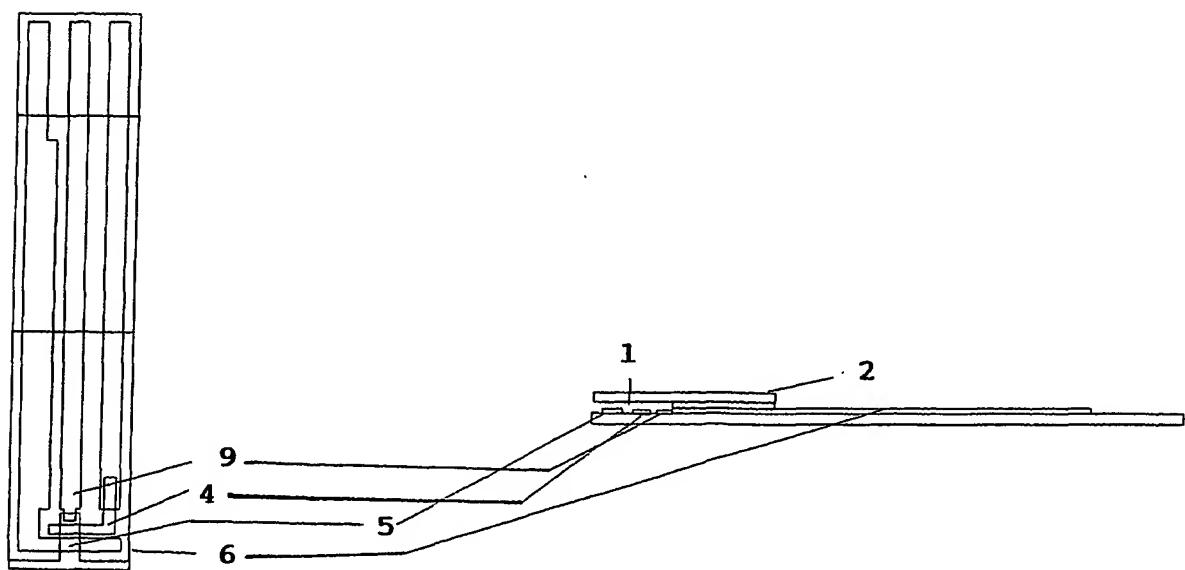
CLAIMS

1. A device for the electrochemical detection of an analyte in a sample, which comprises electrodes and appropriate reagents on a substrate defining a volume within which the sample can be detected, wherein said volume is 500 nL  
5 or less.
2. A device according to claim 1, wherein the volume is 50 to 200 nL.
3. A device according to claim 1 or claim 2, wherein the analyte is glucose.
4. A device according to claim 3, wherein the reagents comprise glucose oxidase.
- 10 5. A device according to claim 3, wherein the reagents comprise glucose dehydrogenase.
6. A device according to claim 4, wherein the reagents comprise ferrocene/ferricyanide.
7. A device according to claim 5, wherein the reagents comprise ferricyanide.  
15
8. A device according to claim 5, wherein the reagents comprise NAD and Meldola Blue.
9. A method for the electrochemical detection of an analyte in a sample, which comprises introducing the sample into the cavity of a device according to  
20 any preceding claim, and detecting the current.
10. A method according to claim 9, wherein the sample is whole blood.

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**Figure 1**

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**Figure 2****Figure 3**

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(43) International Publication Date  
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PCT

(10) International Publication Number  
**WO 02/14535 A3**

(51) International Patent Classification<sup>7</sup>: **G01N 27/327, C12Q 1/00**

(21) International Application Number: **PCT/GB01/03615**

(22) International Filing Date: **13 August 2001 (13.08.2001)**

(25) Filing Language: **English**

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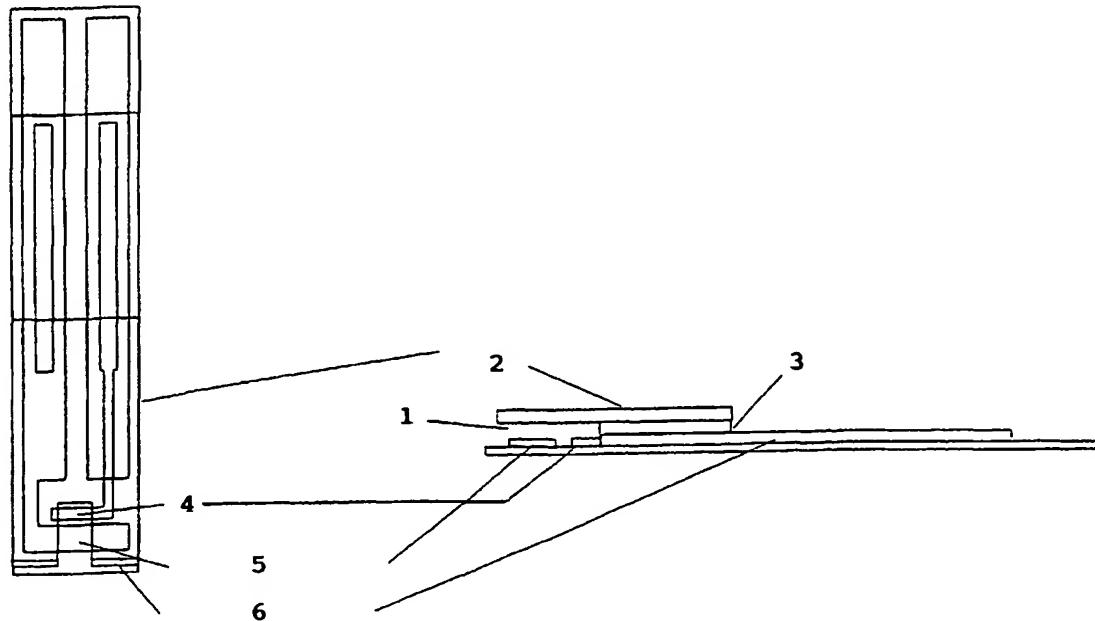
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:  
— *with international search report*

*[Continued on next page]*

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(88) Date of publication of the international search report:

13 June 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 01/03615

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N27/327 C12Q1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 35225 A (TOMASCO MICHAEL F ; HELLER ADAM (US); HELLER E & CO (US); SAY JAMES) 13 August 1998 (1998-08-13) the whole document ---	1-10
X	EP 0 964 059 A (MATSUSHITA ELECTRIC IND CO LTD) 15 December 1999 (1999-12-15) the whole document ---	1-10
X	WO 00 28068 A (CAMBRIDGE SENSORS LTD ; BLAIR NEIL (GB); COX LORNA JEAN (GB); MCCAN) 18 May 2000 (2000-05-18) the whole document ---	1-10
A	WO 97 02487 A (BOEHRINGER MANNHEIM CORP) 23 January 1997 (1997-01-23) abstract ---	1 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

8 February 2002

Date of mailing of the international search report

15/02/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 01/03615

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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**INTERNATIONAL SEARCH REPORT**

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International Application No

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